

Hybridization Detection via *Intensity* Measurement:





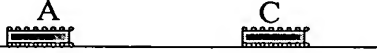


<u>Nucleic Acid Fragments</u>	<u>Fluorescence</u>
Probe A 	= 20 Intensity
Probe B 	= 60 Intensity
Probe C 	= 70 Intensity
Target 	= 80 Intensity
Target 	= 90 Intensity
Target 	= 150 Intensity
Target 	= 130 Intensity

Figure 1.

Figure 4. Analysis of hybridized unitized probe and target.

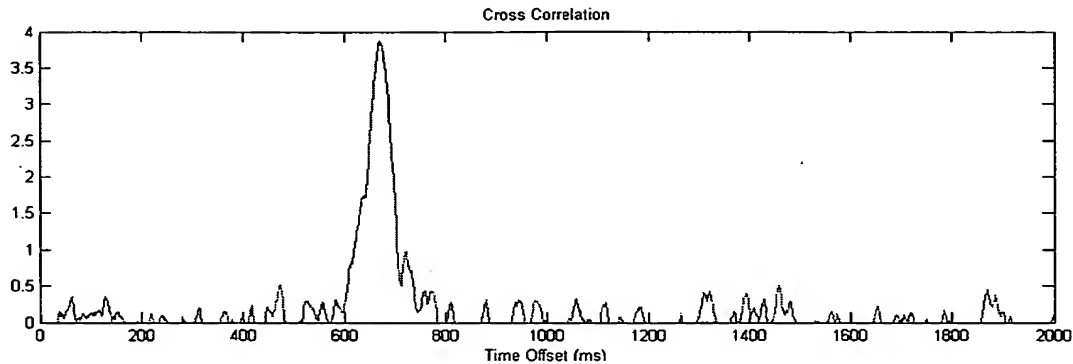


Figure 4a: Labeled PCR product

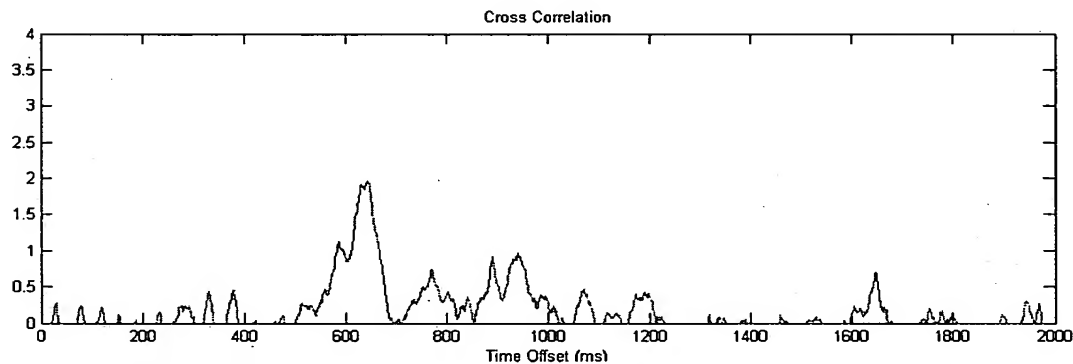


Figure 4b: Labeled PCR product hybridized with 100 fold excess unlabeled PCR product.

Figure 5. Individual molecule analysis – time course of photon burst intensities

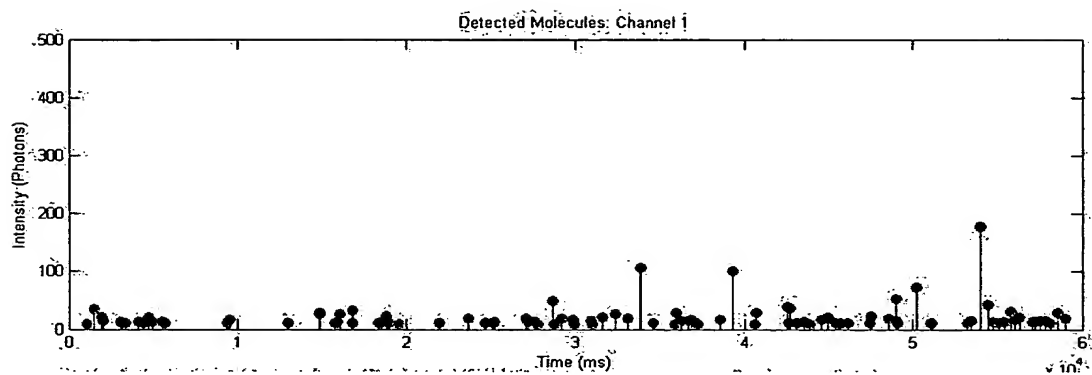


Figure 5a. labeled-unlabeled hybrid molecules

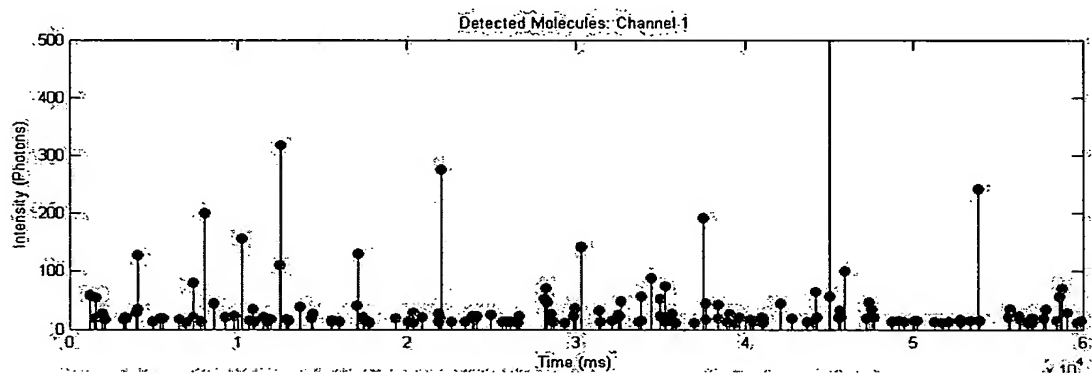


Figure 5b. labeled-only molecules

Figure 6. Histogram of photon intensities of hybridized unitized probe

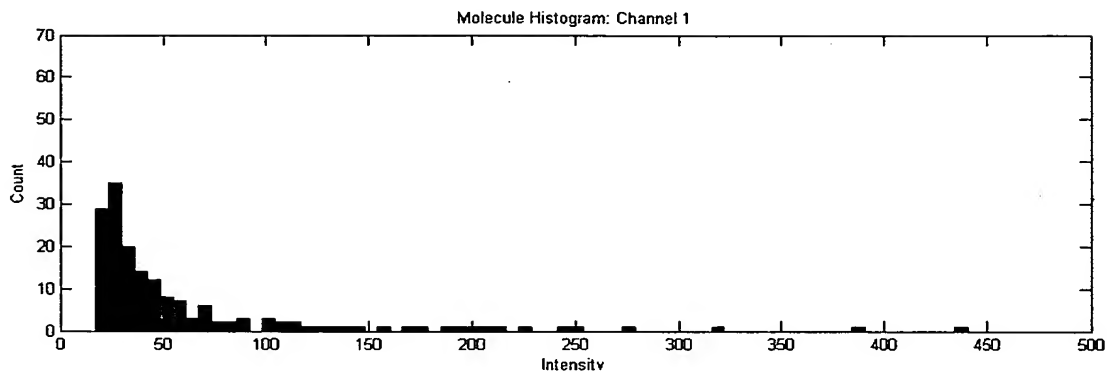


Figure 6a: Labeled PCR product only. 35% of the detected molecules had intensities of greater than 50 photons.

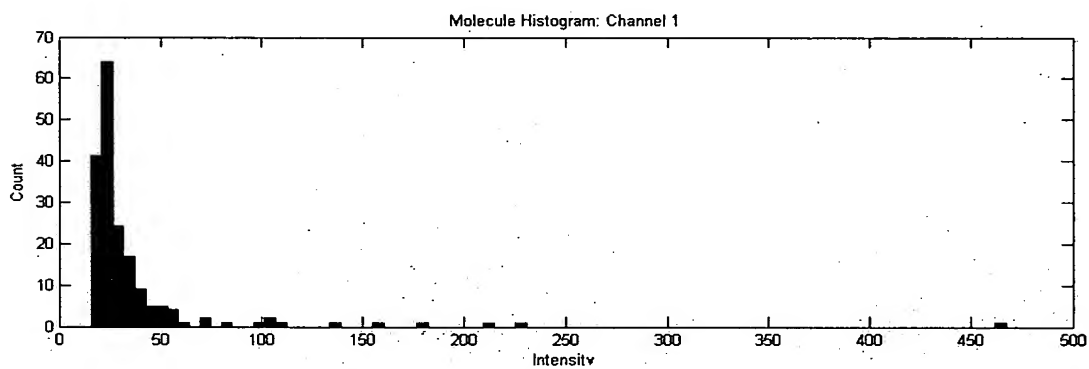


Figure 6b.: Labeled PCR product plus 100 fold excess unlabeled product. 10% of the detected molecules had intensities greater than 50 photons.

Single Molecule Detector

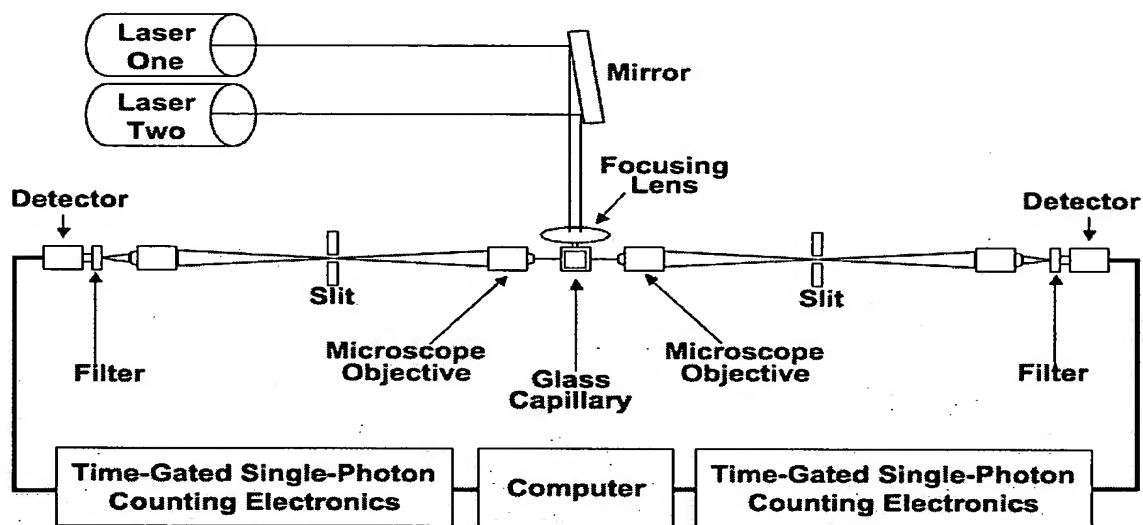


Figure 1 Schematic diagram of the basic apparatus for single molecule detection using laser induced fluorescence.

Heart of the SMD Instrument

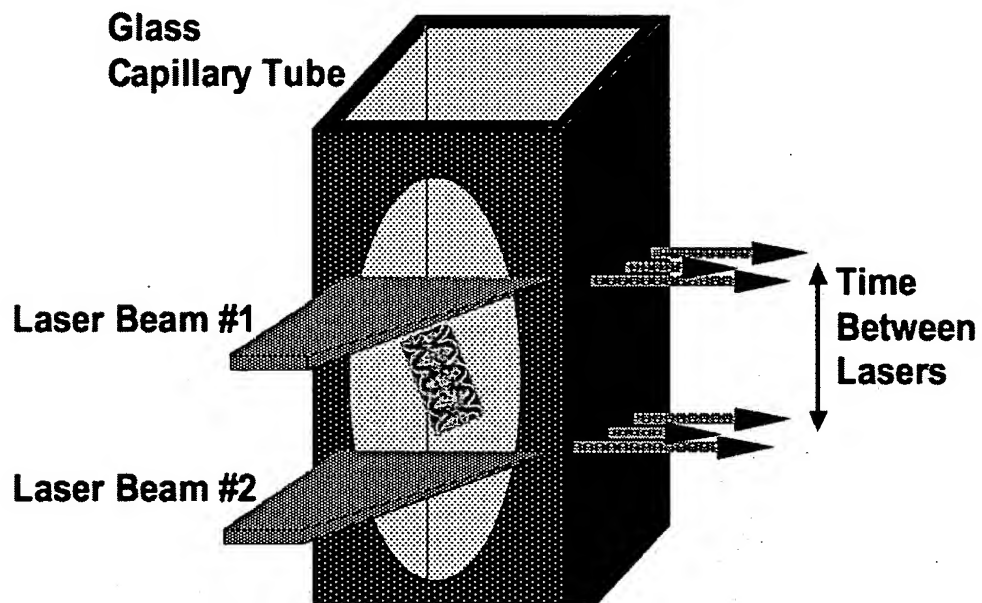


Figure 2. Glass capillary tube forms the heart of the system.

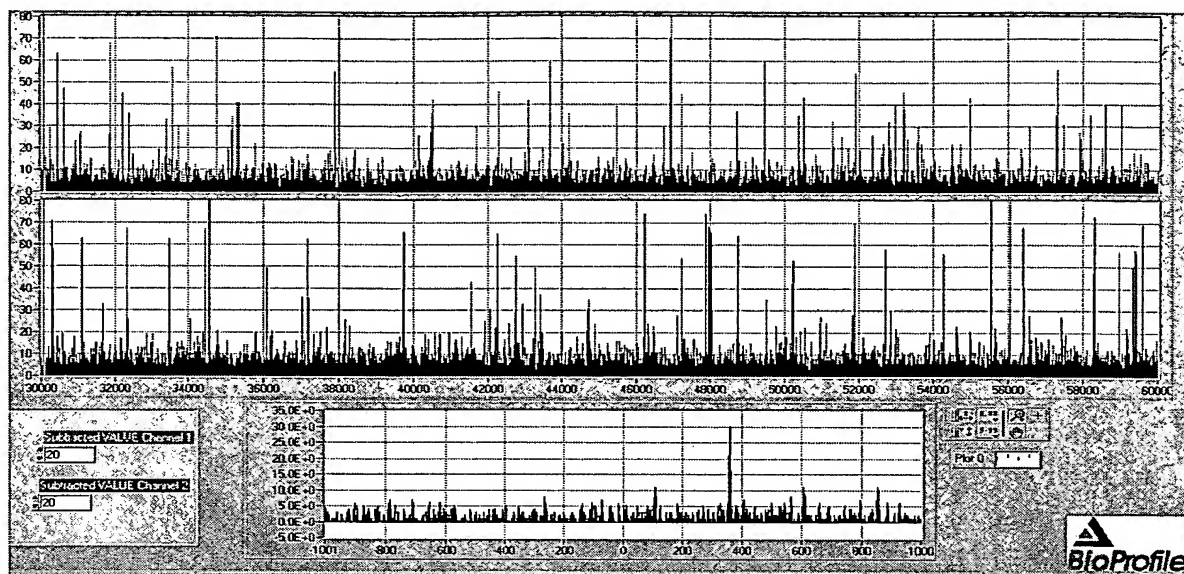


Figure 3. Example of output from existing laboratory device. The upper two traces show the number of photons as a function of time (each unit represents 2 ms) for the two channels. Each large spike represents a fluorescence detection event over the background levels. The bottom trace represents the cross-correlation of the events for channel one with the events for channel two over a 30 sec period with a single peak at 700 msec